membranes by a single glycolipid anchor. But it is also found in lymphoid tissue and, at relatively high concentrations, as a free-floating monomeric protein in blood. This form is not infectious, nor does it seem to cause any harm. It's PrP^{Sc}, the abnormal isoform of PrP, that does the damage. This is the form found associated with the disease, and the infectious agent, which is relatively insoluble and resistant to proteolysis.

How different are PrP^{C} and PrP^{Sc}? Not very. Perhaps they differ by only a minor twist or turn of their polypeptide chains but dogma has it the interchange of PrP^{C} to PrP^{Sc} is self-perpetuating, with twisted PrP^{Sc} serving as a template for the demonic conversion of PrP^{C}. The conversion can be produced in the test tube but it is not autocatalytic *in vitro*, and the 'new PrP^{Sc}' has not yet been shown to be infectious *in vivo*. NMR spectroscopy has given us a picture of PrP^{C} but the structure of PrP^{Sc} and the mechanism of conversion remain unknown.

But it's easy to tell them apart, isn't it? Last year it was simpler. Now, the convenient criteria used to distinguish 'non-infectious' from 'infectious' — relative protease-resistance and insolubility — have been demolished. In one recent study, a 'soluble' PrP^{Sc} of 106 amino-acids was genetically engineered in transgenic mice. In another study, a highly infectious prion strain obtained from a patient with a rare human TSE (Gerstmann–Staussler–Scheinker syndrome) transmitted a TSE-like disease to mice without noticeably changing their normal prion protein. Confused? Join the growing club of people who now doubt the classical description of the infectious agent as 'PrP^{Sc}, a proteinase-resistant protein' and are looking for other factors.

So what does PrP do? Who knows? The protein binds copper and might serve as a copper transport protein in the cellular response to oxidative stress. But it's one of the many proteins that can be 'knocked-out' in transgenic mice without gross adverse effects. The mice eat, drink and mate to their heart's content, although some have trouble sleeping and remembering things … typical research students, actually. But most critically, the mice can't develop prion diseases and they can't replicate the infectious agent. And, yes, someone has patented a PrP knockout cow, with a view to curbing BSE.

Most likely to be mentioned by… Stan Prusiner (of course), Charles Weissmann and Adriano Aguzzi, but the word prion has been shunned by Alan Dickinson, discoverer of the murine prion protein gene, *Sinc*. With Hugh Fraser, he was the first to produce rigorous criteria for typing different strains of sheep scrapie agent. Their criteria are based on the brain pathology and incubation times generated by these scrapie strains in mice of differing *Sinc* genotypes. Their methods were also critical in showing that BSE and new variant CJD are caused by a similar strain of agent. The lack of a molecular explanation for the variety of TSE strains continues to be a thorn in the side of those who believe a single protein could be the infectious agent.

Can we cure new variant CJD or any prion disease? Sadly, no. Pentosan sulphate seems to be the most promising prophylactic agent (in mice, that is) but no compound has yet been effective in humans.

Where can I find out more?

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Primer

The retina

Guilherme Neves and Leon Lagnado

One of the most important challenges for neurobiologists is to understand how neural circuits process and represent information, and one of the most intensively studied neural circuits is the retina. This outpost of the brain carries out the first steps in the conversion of light into vision.

Analyzing a neural circuit involves understanding at least four aspects of the circuit, all of which can be looked at in the retina. First, the information going in and coming out must be clearly defined. Working with the retina, the input (light) can be precisely quantified and manipulated experimentally. Simultaneously, the output that is sent back to the brain can be monitored. Second, the neurons that make up the circuit and their connections must be identified. The retina consists of five classes of neuron with two basic stages of synaptic connection (Figure 1). Third, the signals that pass through the circuit should be measurable. The electrical responses to light have been recorded from all major classes of neuron in the retina and recent advances have made it possible to do this in many neurons simultaneously. Finally, it should be possible to investigate the contribution of individual elements in the circuit in isolation. Most classes of retinal

neuron can be dissociated from the retina and their physiological properties investigated. In addition, transgenic technology has made it possible to selectively ablate a particular class of neuron or knock out a molecule that is essential for a particular function.

Receptive fields and parallel channels

Signals from the retina are sent to the brain along the axons of ganglion cells (Figure 1). These action potentials (also called spikes) can be recorded using an extracellular electrode while shining a spot of light onto the retina. A fundamental observation is that an individual ganglion cell only changes its pattern of activity when light falls on to a restricted region of the retina — known as the receptive field for that particular ganglion cell. The receptive field is a property of all neurons in the retina (and almost all neurons in the visual system) and it results from the fact that the photoreceptors are the only neurons that directly detect light. The size of the receptive field is therefore determined by the distribution of photoreceptors on the retina from which a given cell receives information.

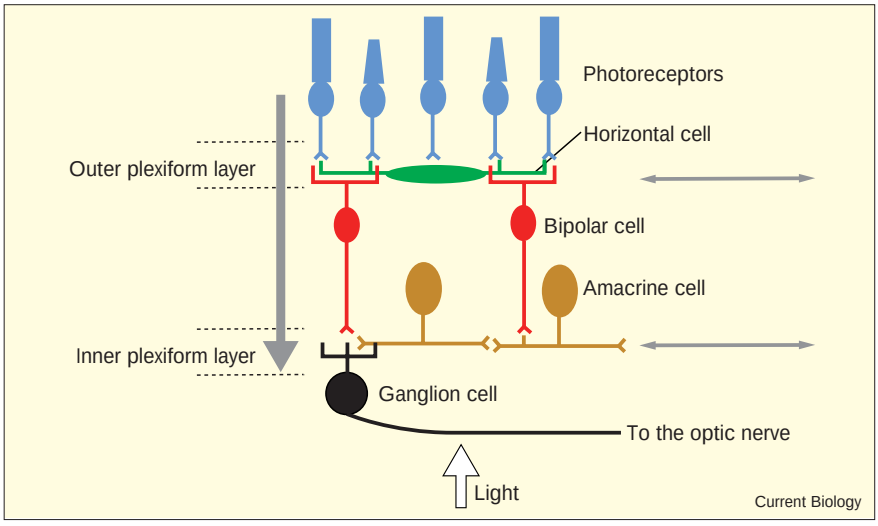
The receptive field of ganglion cells has two regions, a center and a surround, in which light has opposite effects (Figure 2). The response to light in the center defines two types of cell. For so-called 'ON' ganglion cells, light in the center of the receptive field is excitatory, increasing the frequency of the spike discharge, whereas light in the surround is inhibitory (Figure 2a). For 'OFF' ganglion cells, light in the center is inhibitory (Figure 2b). Strikingly, if the whole of the receptive field is illuminated, there is little change in activity — ganglion cells are not interested in uniform illumination. Therefore, an ON ganglion cell behaves as if its job is to detect a bright area on a dark background, whereas an OFF ganglion cell detects a dark area on a bright background. In a natural image, such stimuli would be created by the edges of objects.

The responses of ganglion cells change with time. For instance, when a spot of light was shone onto the center of the receptive field of the ON ganglion cell shown in Figure 2a, at first it responded at high frequency, but then the response weakened (Figure 2a). In many ganglion cells the response to steady light decays away completely, and they respond only when a light goes on or off. Such stimuli would correspond to the edges of objects moving across the retina.

There are roughly the same numbers of ON and OFF ganglion cells, and both types cover the entire surface of the retina. Each photoreceptor indirectly contacts at least one ON and one OFF ganglion cell. The signals from ON and OFF ganglion cells run in distinct neural pathways all the way to the visual cortex in the brain. These ON and OFF 'channels' also run in parallel with other pathways beginning in the retina that are specialized for transferring information about color and movement.

So, the retina employs two basic strategies to extract the most important information from the external world. First, ganglion cells respond best to changes in the visual stimulus, either in space, in time or in spectral composition (color). Second, information is conveyed to the brain in a series of parallel channels, each concentrating on different characteristics of the signal.

Figure 1



Schematic diagram of the retina showing the principal types of neuron and their synaptic connections. Grey arrows indicate directions of information flow. Signals can pass from photoreceptors to ganglion cells through bipolar cells. Signals can also pass laterally, through horizontal cells at the first synaptic stage in the outer plexiform layer, and through amacrine cells in the second synaptic stage in the inner plexiform layer. Horizontal cells and amacrine cells modulate signals transferred across synapses at these stages.

Phototransduction

The first step in vision is phototransduction — the conversion of light into an electrical signal. The photoreceptors that do this come in two varieties: rods and cones. The rods are more sensitive to light but the cones come in three classes that are maximally sensitive to different wavelengths. The phototransduction signal cascade is well understood. The light-sensing molecule in photoreceptors is rhodopsin. When a rhodopsin molecule absorbs a photon of light, a cascade of events is triggered that amplifies the signal. First, the photoactivated rhodopsin

activates hundreds of copies of transducin, a trimeric G protein. Having bound GTP, the α subunit of transducin activates a cGMP phosphodiesterase. So the photon causes a decrease in the levels of cGMP in the cytoplasm of the photoreceptor. The plasma membrane contains cation channels that are opened by cGMP from the cytoplasmic side, and hundreds of these channels close in response to light. The resulting block of inward current flow causes the inside of the photoreceptor to become more negatively-charged (hyperpolarized), which in turn leads to the closure of calcium channels in the synaptic terminal of the photoreceptor and a decrease in the release of the neurotransmitter glutamate. In other words, light reduces glutamate release from photoreceptors.

Adaptation

The retina functions at light intensities spanning ten orders of magnitude (from starlight to bright sunshine). Rods respond to low light levels and cones to higher levels. The range of light intensities over which rods and cones can respond is extended by a process of automatic 'gain control' (similar to that in a video camera). This process is called adaptation. When the intensity of the light is increased, the amplification of the phototransduction cascade is reduced. The negative feedback signal is a fall in the free-calcium concentration in the cytoplasm that acts on several proteins in the phototransduction cascade.

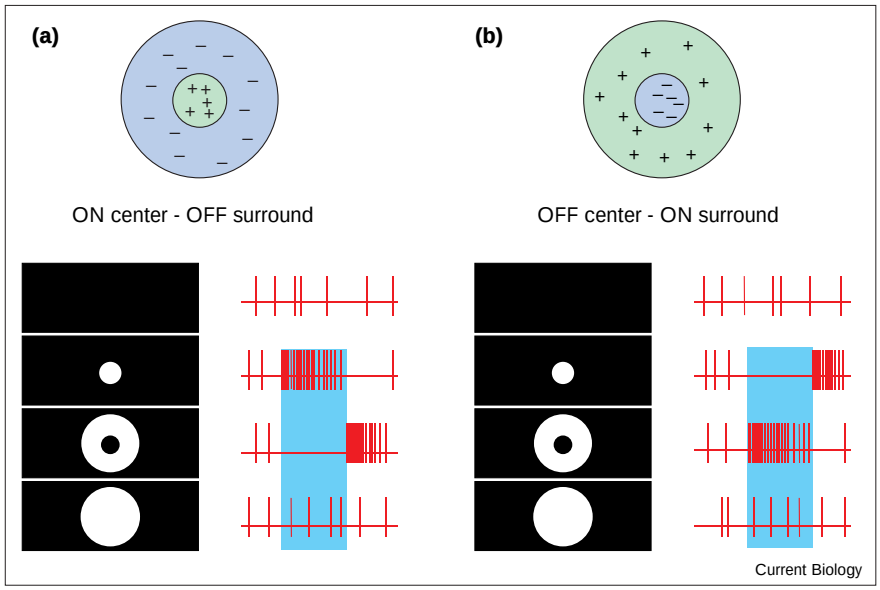
Ganglion cells adapt at light intensities even lower than the rods, indicating that the sensitivity of the retinal circuit can be altered independently of the photoreceptors. But the retina is even cleverer than that — it learns. The way it processes information depends on recent experience. For instance, the sensitivity of the retina to contrast is regulated. It seems that synaptic connections alter on the time-scale of seconds to minutes, and this involves both structural and electrical changes in the neurons. Some of these changes are evoked by dopamine, a neuromodulator released by amacrine cells.

Wiring a receptive field

ON and OFF channels and center–surround receptive fields do not originate in ganglion cells; they start in bipolar cells. In the center of the receptive field of an OFF bipolar cell, glutamate released by photoreceptors directly opens non-specific cation channels that depolarize the bipolar cell. So, because light reduces glutamate release from photoreceptors, OFF bipolar cells hyperpolarize in response to light (Figure 3). ON bipolar cells contain a glutamate receptor that acts similarly to rhodopsin; it couples to a G protein and cGMP phosphodiesterase, and glutamate binding results in the closure of cGMP-gated channels. Light (which reduces levels of glutamate) therefore opens these channels to depolarize the ON bipolar cell. The amplifying cascade helps the reliable transmission of signals as small as a single photon. Transgenic mice that do not express this glutamate receptor are unable to generate ON responses.

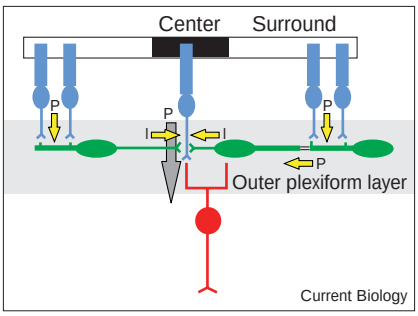
The surround of the receptive field of a bipolar cell is generated by photoreceptor signals that reach it indirectly, through horizontal cells that extend laterally across the retina (Figure 3). Horizontal cells release the inhibitory neurotransmitter GABA onto the synaptic terminals of photoreceptors. Light hyperpolarizes horizontal cells and reduces GABA release, causing the photoreceptor to depolarize. Horizontal cells are electrically connected through gap junctions, allowing signals to travel relatively large distances across the retina. Light closes gap junctions and makes the receptive field shrink. This effect is mediated by dopamine released from amacrine cells.

Figure 2



Center–surround receptive fields in ON and OFF ganglion cells. (a) Top: map of responses of a ganglion cell to a small spot of light, showing locations where the spot increased (+) and decreased (–) the firing rate of this cell. Bottom: spike trains (red) elicited by four different stimuli. These are (from top): darkness; spot of light in center of receptive field; annulus of light in surround; and uniform illumination of whole receptive field. The blue region shows the timing of the stimulus. (b) Responses in an OFF ganglion cell to the same stimuli shown in (a).

Figure 3



Generation of a center–surround receptive field in an OFF bipolar cell. The center is generated by direct connections (grey arrow) from photoreceptors (blue) to a bipolar cell (red). The synapse is sign-preserving (denoted by P), that is to say, a hyperpolarization in the pre-synaptic photoreceptor is transmitted as a hyperpolarization in the post-synaptic bipolar cell when light illuminates the center. The surround is generated by signal flow, shown by yellow arrows. When the surround is illuminated (as shown), photoreceptors cause horizontal cells (green) to hyperpolarize through sign-preserving synapses that release glutamate. Signals in horizontal cells spread laterally through gap junctions. Photoreceptors in the center receive inputs from horizontal cells but because the transmitter involved, GABA, is inhibitory, the synapse is sign-inverting (I). Photoreceptors in the center therefore depolarize when the surround is illuminated, antagonizing the effect of light in the center.

Photoreceptors, bipolar cells and horizontal cells in the outer retina do not fire action potentials; they generate relatively sustained voltage changes that are graded with light intensity. The signals in amacrine cells and ganglion cells in the inner retina differ in two ways: they generate action potentials, and many respond transiently when a light goes on or off. The receptive field of a ganglion cell is derived from the bipolar cells that provide its inputs. The time-course of the response is strongly affected by amacrine cells, which have a key role in signalling change and are very sensitive to moving stimuli. Amacrine cells release GABA onto the terminals of bipolar cells to control synaptic transmission to ganglion cells. Amacrine cells are electrically coupled, and these gap junctions, like those between horizontal cells, are modulated by dopamine.

Future directions

Most work on the retina, or any other neural circuit, has measured the output one neuron at the time, but it has recently become possible to record the responses of many ganglion cells simultaneously. These measurements reveal that ganglion cells do not respond independently of one another, and that information is contained in the pattern of spikes emerging from different cells. To understand vision, we must decipher this multi-neuronal code. To understand how this code is generated, we must understand how synapses transfer information, and how this transfer can be modulated.

Adaptive changes in the way the retina processes information will involve changes in the synaptic connections. Recently, it has become possible to make direct measurements of the processes regulating neurotransmitter release at the synapse of isolated retinal neurons, and in the future we can hope to understand these processes at the molecular level. Structural changes in synaptic connections are only just beginning to be characterized, and it will be fascinating to understand how these alter the flow of signals.

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Vacuolation in murine prion disease: an informative artifact

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Vacuolation is the spongiform change in the brain that is regarded as one of the pathological hallmarks of the prion diseases. Other hallmarks include astrocytosis, amyloid deposition and loss of neurons. Vacuoles vary in size from 5 to 15 μm diameter and the consensus opinion is that they are located within dendrites and axons [1,2]. A description of the density and distribution of vacuoles is central to the definition of the 'lesion profile', which has been used to characterise the different strains of scrapie agent [3] and, more recently, to verify that the prion agent in bovine spongiform encephalopathy is similar to that found in the new-variant Creutzfeldt–Jakob disease [4]. The accumulation of scrapie infectivity has been shown to precede the development of vacuolation [5,6]. Although some studies have reported a direct correlation between the level of infectivity and the severity of vacuolation [7], others have not shown this [8]. The accumulation of the scrapie form of the prion protein, PrP^{Sc}, as shown by immunocytochemistry, has been reported to occur at an early stage of prion disease in mice, and has been shown to precede the development of vacuolation [9].

The molecular mechanisms responsible for vacuolation and the significance of vacuolar pathology in the prion diseases remain poorly defined. Jeffrey and colleagues [2] hypothesise that vacuolation is a non-specific spongiform change,